

Observations on Evisceration and Visceral Regeneration in the Sea-cucumber, *Actinopyga agassizi* Selenka

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(Plates I & II; Text-figures 1 & 2)

INTRODUCTION

IT is well known that a number of holothurians autotomize their viscera under various conditions, and it is generally believed that complete regeneration takes place until the reformed organs closely resemble their normal counterparts. The general interpretation of the process has been that this is an action of defense, the expelled viscera entangling a potential enemy, and also that it may permit the survival of the holothurian by reducing the need for oxygen under adverse, suffocating conditions. The aspidochirote holothurian, *Actinopyga agassizi* Selenka, is one of the commonest shallow water species found in the Bahama Islands area. This sea-cucumber can be relatively easily induced to eviscerate and it contains a large and conspicuous organ of Cuvier. A study of this animal has been made in order to determine the gross pattern and mechanics of the reformation of the intestine, together with some of its histological features. A number of preliminary experiments were performed in an attempt to discover the factors stimulating evisceration and to elucidate the role of the organ of Cuvier in the light of recent information on its toxicity, reported by Nigrelli (1952) and Nigrelli & Zahl (1952). I wish to acknowledge the kind support and assistance of Mr. William S. Glazier who made it possible for a large portion of this work to be carried out. My thanks are due Mrs. Louise Palmer Wilson for her advice and suggestions towards the completion of the histological studies made at Wellesley College. A portion of this work was carried out at the Lerner Marine Laboratory, Bimini, Bahama Islands. I wish to acknowledge the great assistance of Dr. Charles M. Breder, Jr., in the development of the diagrammatic presentations included herein.

EVISCKERATION

Experimental Induction

The striking phenomenon of self-mutilation which occurs widely throughout the orders of Holothuroidea has drawn the attention of many investigators. The manner and extent of self-mutilation appear to be similar among all of the large aspidochirote holothurians in which it has been studied. Evisceration is effected by a rupture through the cloaca of almost the entire alimentary canal with its associated glands, some of the gonad tubules, and in some species, one or both of the respiratory trees. The organ of Cuvier is associated with the respiratory trees of some species and is apparently linked in some way with the phenomenon of evisceration. The occurrence of this organ, however, is extremely variable, even within a single genus (Deichman, 1930), so that its role is not one indispensable to total evisceration. A number of species of the genus *Holothuria* have a large, conspicuous organ of Cuvier which becomes a mass of sticky threads on contact with sea water. The general interpretation is that the expulsion of this organ is associated in some special way with evisceration and precedes the more radical defense response of autotomy of the digestive system. In his studies of *Holothuria nigra*, Hérourard (1889) reported that the expulsion and elongation of the tubules of the organ of Cuvier were results of water being forced into their lumina from the left respiratory tree. Mines (1912) supported these observations and like Hérourard, expressed the view that the Cuvierian organ played no specific role in evisceration, but rather that it was expelled before the viscera simply as a result of its anatomical proximity to the cloaca. On the other hand, Minchin (1892) reported an intrinsic extending apparatus in the Cuvierian

organ of *H. nigra* and expressed his view that the organ was specialized for expulsion, probably in the role of a defense organ. Crozier (1915) reported a similar intrinsic extending apparatus in the organ of *H. parvula* (= *H. captiva*). The interpretation of the response of total evisceration in different species varies widely. Bertolini (1930) believed that in *Stichopus regalis* it is a naturally occurring seasonal phenomenon. Minchin (1892) took a similar view of *Holothuria nigra*. In contrast to this, it has been reported that evisceration of *H. parvula* is a result of adverse conditions rarely occurring in nature (Crozier, 1915).

The Cuvierian organ of *Actinopyga agassizi* seems to be in some way involved in total evisceration. As described for species of the genus *Holothuria*, the organ of *Actinopyga agassizi* may be everted but not eliminated without evisceration occurring, but evisceration never occurs without the previous everting of the organ of Cuvier. It appears to react to the same stimulus which induces autotomy, or to be a factor leading up to expulsion of the viscera. In the majority of cases, the organ is expelled within the first half hour under experimental conditions, although generally it is not completely eliminated, maintaining its attachment to the respiratory trunk while hanging free outside the body cavity. It is not an immediate preliminary to total evisceration, for in many cases the animal, having thrown out the organ of Cuvier within the first half hour, will not eviscerate totally for five or six hours. At the time of evisceration, if the Cuvierian organ still maintains its attachment to the respiratory trunk, it appears to be a matter of chance whether or not it is torn free by the expulsion of the alimentary tract.

A variety of methods was tried for stimulating evisceration in *Actinopyga agassizi*. With all of them the length of time before evisceration did not seem a significant factor for comparison, because within any given method the time before evisceration varied to a great extent from one animal to another. Freshly collected animals were used in all cases, rather than those which had been kept in stock tanks.

The most effective method appeared to be to allow a number of animals to remain in standing water in a galvanized container exposed to the sun, so that the water was heated to a relatively high temperature. The majority of such animals eviscerated within one-half to seven hours and there was almost 100% survival during the experimental regeneration period. In an attempt to single out a particular condition in this general method responsible for stimulating evisceration, three controllable factors were

tested: temperature, trace metallic ions and oxygen deficiency.

To avoid contamination with trace metallic ions present in galvanized containers, a number of animals were kept in enamel pails or glass bowls. Three were allowed to stand in enamel pails at a temperature varying from 20° to 25° C. One of these holothurians showed no sign of evisceration in six hours. However, when it was removed to fresh running sea water, it eviscerated immediately. Two animals were left in enamel pails for 24 hours and showed no indication of evisceration at the end of this period. Subsequently they were put together in a galvanized tub, at about 20° C., and they eviscerated in four to ten hours. One animal, maintained in an enamel pail at a temperature which was raised to 40° C. and allowed to range down to 33° C., eviscerated in the first three hours. Two others were placed in glass bowls in about two liters of water at a temperature of 35° C. They eviscerated in five hours. During the same period of time, four animals in water of 20°-22° C. in a galvanized tub did not eviscerate. One of these subsequently eviscerated upon being moved to an aquarium with a supply of running sea water.

Freshly collected animals were placed in large glass bowls in two liters of water at about 22° C. and to each a metallic chloride was added—zinc to the first, copper to the second and iron to the third. A precipitate formed when each salt was added to sea water. The animal treated with zinc eviscerated after five hours, whereas the others, including a control to which no salt was added, did not eviscerate for a much longer period. After 17 hours, both the control animal and the one treated with copper ion had eviscerated, but the one treated with iron showed no effect other than expulsion of the Cuvierian organ in the first half hour of treatment, as did the other experimentals and the control.

The results of these tests are somewhat contradictory and puzzling. In addition, casual observation indicates that many animals eviscerate for no readily apparent reason. If they are kept undisturbed in aquaria with no detectable change in environment, after about a week one may eviscerate while another in the same aquarium will not.

It appears, however, that evisceration of this sea-cucumber occurs but rarely, if at all, in nature. Expelled viscera are never observed and animals found with incomplete digestive systems have not been reported. Such factors as the presence of metallic ions, especially zinc, and high temperature evidently play an important role in the stimulation of evisceration, but no clear-cut pattern can be established by these experiments.

It is possible that some adverse condition was common to all the methods used, or that the same reaction by the animal can be brought on by several different factors. It is interesting to note here that Kille (1936) reported being able to induce evisceration by means of electrical stimulation.

Role of the Organ of Cuvier

The exact role of the organ of Cuvier in evisceration remains a problem. Whether its behavior is only a part of the response to conditions which induce autotomy, or whether it is the total response, in its turn creating conditions inducing autotomy, remains obscure. Recent investigations by both Nigrelli and Zahl on the toxic factors of *Actinopyga agassizi* have presented a rather different view. The organ of this species is not extremely sticky, although it is ejected in much the same way when the animal is disturbed as has been described for species of *Holothuria*. In his analysis of the various organs of the sea-cucumber, Nigrelli (1952) determined that the organ of Cuvier contained an active toxic agent. The water soluble factor extracted from this organ proved intensely toxic to both fish and mice. The organ of Cuvier is generally considered a modification of the respiratory system, an outgrowth at the junction of the left respiratory tree with the main trunk. The organ in certain species has been described as tubular in structure, although this has not been found to be the case in *Actinopyga agassizi*. In many species of holothurians in which a large Cuvierian organ is present, these tubules, when expelled, become excessively sticky. It seems to be because of this property, that authors have concluded that the organ is specialized for defense, being thrown out to entangle a potential enemy.

To determine the nature of the organ of Cuvier, a variety of tests with living animals and a histological study of the organ itself were made. For testing the toxic factor, *Cyprinodon baconi* Breder was the fish used. It takes but little stimulation of the sea-cucumbers to cause the release of the toxic substance. Merely by picking one of the animals out of an aquarium, rubbing it slightly and replacing it, enough poison is released in the water of a 15-gallon tank to kill fish within less than half an hour. The most apparent observable result of rubbing a live animal in this way is the secretion of a quantity of mucus all over the surface of the body.

In order to determine the source of the released toxin, sea-cucumbers were kept in aquaria with running sea water until there was no contamination in the tank—usually a matter of 24 hours. Fish were put in these tanks several hours before the test to check the absence of toxicity in

the water. Subsequently, the holothurians were picked out of the tanks and samples of the skin mucus were collected in beakers as it accumulated and dripped off the body wall. After the animal was held for several minutes, there was a strong expulsion of water from the cloaca. This water was collected in beakers held at a distance from the anal opening to prevent possible contamination by the skin mucus dripping down from the test. To determine the toxic potency of these samples, test fish were established in glass bowls in two liters of sea water. It was found that when a sample as large as 10 cc., collected from the cloaca as described above, was added to the two liters of sea water in a glass bowl, the test fish survived for a period of at least four hours, at which time the test was discontinued. In contrast to this, only an hour after addition of a few drops of the collected mucus secretion, the test fish died in the violent spasms typical of fish poisoned with holothurin.

To determine the effect of removal of the Cuvierian organ on toxin production, two animals were induced to eviscerate, with an attempt to eliminate the whole organ. Following evisceration each was maintained for five days in a 15-gallon tank supplied with running sea water. Throughout this period, the animals were disturbed once a day until mucus skin secretion clouded the tank. As standard procedure, fish were introduced into the tanks before the holothurians were disturbed to check the absence of residual poison from previous tests. At the end of five days, the sea-cucumbers whenever disturbed proved as effective in killing the test fish as animals retaining the Cuvierian organ. Dissection showed no trace of this organ in either of the two test animals.

A normal sea-cucumber was maintained in a 15-gallon aquarium with running water for a period of six days, during which it was daily disturbed and handled until sufficient skin mucus was secreted to cloud the tank. Test fish were introduced into the tank an hour or more before the animal was disturbed each day to confirm the absence of contamination from the previous day. It was found that, although the tank water was toxic to these fish up to 12 hours after contamination, it was generally non-toxic at the end of 24 hours. The sea-cucumber showed no adverse effects of being handled so extensively, although generally an animal when disturbed to such an extent will eviscerate readily. When it was killed and dissected at the end of six days, a pearl fish, *Carapus bermudensis*, was found in one of the respiratory trees. The fish showed no deterioration whatsoever and it is probable that it was killed only on fixation of the sea-cucumber. The obvious question arises as to how

this fish survived the week of constant poisoning, in which the host was pumping highly toxic aquarium water into its respiratory system. In preliminary tests, Dr. L. Aronson (*personal communication*) found that pearl fish exposed with several *Cyprinodon* to the toxin in a tank clouded with mucus skin secretion of *Actinopyga agassizi* were affected immediately, although they survived about three times as long as the *Cyprinodon*. Nigrelli (1952) reports that a dilution of 1:100,000 of a standard stock solution of Cuvierian organ extract killed *Cyprinodon* in 23 minutes; whereas a dilution of 1:1,000 killed pearl fish in eight minutes. This single instance of survival of a pearl fish within the respiratory system suggests the possibility of some mechanism which protects the sea-cucumber itself from the highly toxic substance released by the skin glands, and which, incidentally in this case, evidently protected the pearl fish.

Sections made of the Cuvierian "tubules" do not present the picture described in such detail for the organ of Cuvier of other holothurian species. Although Jourdan (1883), Cuénot (1891), Guislain (1953), Hérouard (1889), Minchin (1892) and Mines (1912) have described these structures as tubules in *H. nigra* and *H. impatiens*, they are clearly not tubules in *A. agassizi*. In sections of the cloaca and the base of the respiratory trees, no lumen could be traced out from these structures other than the typically constructed branches of the respiratory system. In preparations of the Cuvierian organ itself (Pl. I, Fig. 1), there is no central canal, and no evidence of epithelial cells in the central area of the rod which might indicate a collapsed lumen. On the contrary, the central area appears to be a core of connective tissue. The tissue surrounding the central core is not of a secretory nature, but rather gives the appearance of a storage tissue. It is highly vacuolated, the vacuoles being surrounded by fine cytoplasmic strands containing two or three nuclei. Halfway out to the periphery from the central connective tissue core, there are both circular and longitudinal muscle strands. In the sections studied, prepared by standard alcohol techniques, the vacuoles appeared empty.

REGENERATION

Morphological Aspects

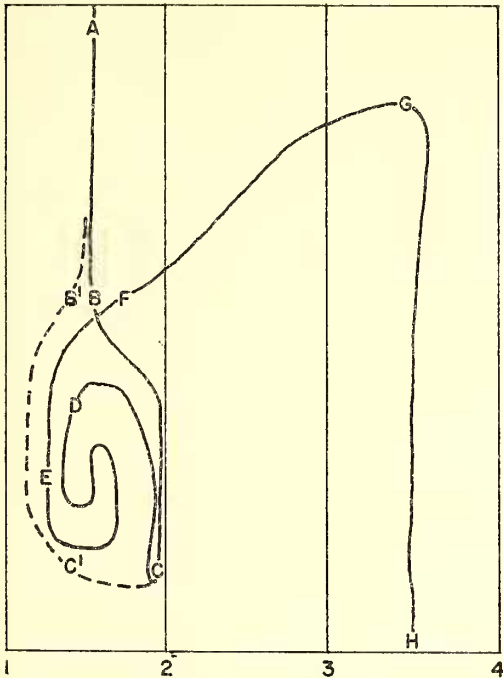
In the study of regeneration of tissue that has been either mutilated or completely discarded in evisceration, an effort was made both to discern the gross plan together with the mechanics of the reformation and to determine the histology of the newly differentiated tissues and organs. Following evisceration, the animals were maintained either in the sea in wire cages permitting

free flow of water, or in aquaria with a supply of running sea water. In these two environments, the temperature ranges differed to some extent: in the cage the range was approximately 30°-33° C., while in aquaria it was 20°-24° C. The two sets of experimental animals kept in different environments present somewhat different regeneration schedules, but these schedules vary in time rather than in sequence. Following evisceration, animals were sacrificed at intervals of 1 to 31 days. They were killed by injecting into the body cavity either formalin or Bouin's fluid, followed by immersion of the whole animal in the fixing fluid. No dissection was done until the specimen had been thoroughly fixed and the tissues hardened to some extent. Bouin's fluid was found to be by far the more successful fixative.

The general plan of the alimentary tract is diagrammatically represented in Text-fig. 1, with labelled reference points which will be used to relate the regenerating structures. The essential course of the intestine is shown, omitting the numerous convolutions into which it falls, especially from B to H. The lettered points relate both to the course of the intestine and to the base of the supporting mesentery where it is attached to the coelomic wall. In the area B-C, the intestine and attachment to the coelomic wall do not follow similar paths. The path followed by the latter is indicated by the broken line, the connecting mesentery conforming in a complicated manner between the region C-F.

In specimens killed very shortly after evisceration, the coelomic cavity appeared to be almost an empty shell, containing only extreme anterior and posterior fragments of the intestine, the respiratory trees and the cloaca. The variation of extent of evisceration affected only the gonad tubules and the organ of Cuvier. The evisceration of the gonad tubules was extremely variable and apparently depended solely on mechanical conditions, the extent of their entanglement with the intestine and its associated glands seeming to determine their fate, *i. e.*, either evisceration or retention within the body cavity. To some extent, the retention or evisceration of the organ of Cuvier appeared to depend on a similar mechanical condition. However, it seems that this organ plays a more active role in evisceration, a role that is not yet adequately understood. Whereas it was the usual occurrence for this organ to be thrown out through the cloaca long before total evisceration, it appeared to be a matter of chance whether it was torn free from its attachment to the respiratory system or whether it maintained this attachment and was withdrawn into the body cavity.

Despite the total elimination of the intestine and its mass of associated glands, the track of



TEXT-FIG. 1. Diagram of part of the coelomic wall cut and spread to form a flat surface. The perpendicular guide lines represent four of the five radii. 1, right dorsal radius; 2, left dorsal radius; 3, left radius; 4, ventral radius.

The curved solid line represents the path of the intestine from its junction with the stomach, A, to the point at which it empties into the cloaca, H. This line also represents the path of the attachment to the coelomic wall of the supporting mesentery, except from B-C. There the broken line B'-C' represents the base of the mesentery where it is attached to the coelomic wall.

the original alimentary canal could be traced and the reference points readily identified by the torn supporting mesentery. In the first three days following evisceration, it was the growth and reformation of the mesentery alone that could be followed by gross observation. Following the autotomy of the viscera, this appeared only as a very narrow flap of tissue, much torn at its free edge. The following stages were established arbitrarily on the basis of observation of gross changes of the reforming mesentery and intestine. The letters denoting the area of the mesentery and intestine rudiment refer throughout to those reference points marked in Text-fig. 1.

STAGE 1: The stomach, broken at its junction with the intestine during evisceration, appears as a tube opening into the coelom. Just posterior to the end of this tube, the dorsal mesentery A-C

is much reduced in width, presenting only a narrow flap of tissue, with a very irregular free edge. The course of this mesentery may be traced through its convolutions, C-F, posteriorly to the level of the cloaca, H, and it presents a picture identical with that of the mesentery base found in normal animals. Because the mesentery has been sheared off close to its attachment to the coelomic wall, the portion B'-C' does not extend across D-F, but lies as a narrow fringe to the left of this area. In no area does the mesentery appear to have been completely discarded during evisceration, but merely torn close to its attached edge. At the level of the cloaca, H, there may be a short segment of the posterior intestine, varying in length, which appears as an open tube into the coelom very similar to the anterior tube of the stomach.

STAGE 2: The open end of the stomach tube has a gathered appearance about its circumference, reducing the opening. In specimens in which a remnant of the posterior intestine is retained through evisceration, the anterior extremity of this remnant is closed. The most striking feature of early regeneration is the great extension in width of the supporting mesentery, especially along its length from A to G. Because of its spiral course, B-F, parts of the widening mesentery overlap other parts. There is not such extreme widening of the mesentery from G to H, but here the free edge of the mesentery is longer than its base so that the narrow fringe becomes much folded upon itself laterally, giving a ruffled appearance.

STAGE 3: The posterior tip of the stomach tube is closed. There is further widening of the mesenteries. The length of the mesentery G-H is extremely folded upon itself laterally, and its free edge gives the appearance of having been pulled together by a drawstring. There is a very fine, rod-like thickening along the free edge of this part of the mesentery.

STAGE 4: A fine, thread-like thickening is apparent along the free edge of the mesentery posterior to the stomach, and the broad part of the mesentery B-F supports a relatively short, thick intestine rudiment. The part of the mesentery originating along B'-C' has widened sufficiently to permit the rudiment which it supports to lie almost directly over the area D-E. This enlarged rudiment, lying midway between B'-C' and B-C, curves towards the left dorsal radius (Guide line no. 2, Text-fig. 1), continuing a curving path until it almost completes a circle, then turns back on itself towards F as it tapers to a fine thread. There is a lumen present in this enlarged curving segment of intestine rudiment although in both its anterior and distal extremities the rudiment consists only of a fine solid rod.

STAGE 5: Immediately following the formation and enlargement of the rudiment in the dorsal interradius, there is a further widening of the mesentery B'-F, permitting the formed rudiment to follow a more simplified path. The enlarged curved portion of the rudiment now turns sharply, forming a cusp, and passes under the more proximal part. There is a general increase in diameter of the rod-like rudiment along the mesentery A-B, tapered so that the anterior part of the rudiment is of smaller diameter than the enlarged curved part of the rudiment B-F. The diameter of the rudiment F-G has also increased, tapering to a fine thread at G. There is little observable change in the area G-H, except for a small increase in width along the length of the mesentery and some slight increase in the diameter of the intestine rudiment.

STAGE 6: There is a continuous lumen from the stomach, through the intestine rudiment of the curve, but not extending beyond F. Distal to F, the rudiment remains as a solid rod along the edge of the mesentery.

STAGE 7: There is now a period in which there does not appear to be any great extension in total length of the rudimentary intestine. Rather there is continuation of the development of the existing rudiment, which increases in its diameter along its entire length, and the extension of a lumen through the posterior intestine until the lumen is continuous from stomach to cloaca.

STAGE 8: Once the lumen is continuous throughout the rudiment, there follows a period of growth in which the intestine increases extensively in length. There is extensive lengthening in the curved portion of the rudiment B-F, forming a loop of intestine which extends posteriorly almost to the level of the cloaca before curving anteriorly and ventrally towards F. This loop does not follow the double spiral, B-F, but simply has a descending and ascending limb. The descending limb depends from a wide part of the mesentery, B-C, and thus is permitted to extend across and obscures the ascending limb as far as F.

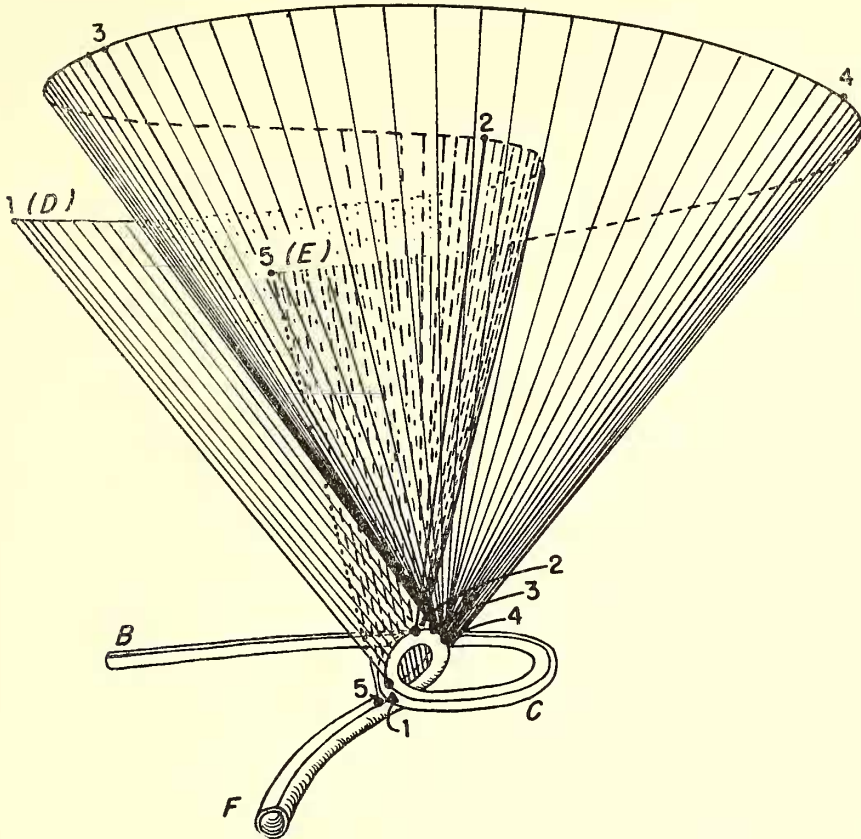
The posterior intestine, G-H, is extensively lengthened, having carried its previously folded mesentery out into a smooth sheet which follows the convolutions into which the intestine rudiment falls. This longitudinal growth of the intestine continued through 31 days of regeneration, the period at which the last specimen was sacrificed. At this time, the reformed intestine was still considerably shorter than in the normal animal, especially in the curving area B-F, and in the posterior area G-H.

In the group of animals regenerating at higher temperature, the initial development was ex-

tremely rapid. In this group, 4-, 5- and 6-day specimens correspond to Stages 4, 5 and 6. One specimen, having regenerated for only 18 days, had developed a continuous lumen from stomach to cloaca that was, at the time of fixation, at least mechanically functional. The group of animals maintained at lower temperature did not develop so rapidly; a 10-day specimen was only at Stage 5 of regeneration.

The general plan of regeneration is the formation of an intestine rudiment along all of the ragged free edge of the remaining mesentery. However, because of the double spiral which the mesentery forms, B-F, and because of differential growth rates of areas of this part of the mesentery, the convolutions of the developing intestine rudiment in this area do not retain a fixed course, but go through a gradual change as development proceeds. Text-fig. 2 shows the relationship between the mesentery and the rudiment found at Stage 4. Whereas the mesentery represented corresponds to D-E the regenerating tube shown corresponds to the area B-F of Text-fig. 1. This illustrates very well one of the basic complicating factors in the mesentery-rudiment relationship. In the beginning stages of regeneration, the rudiment formed is very much shorter than the normal intestine, yet it is supported by the existing mesentery. The formed rudiment does not bypass any part of this existing mesentery but follows its free edge throughout. In the early stages of regeneration, the mesentery widens extensively, but at the same time its free edge becomes very much shorter than its base on the coelomic wall, permitting attachment to the short rudiment.

In order to understand the relation between the curving path of the formed rudiment and that of the normal intestine and to grasp the progressive development which permits the path of the former to approach the path of the latter, it is necessary to compare closely the two diagrams of Text-figs. 1 and 2. The rudiment B-C in Text-fig. 2 does not follow the path of the normal intestine but rather lies close to broken line B'-C' in Text-fig. 1. Because its supporting mesentery is still relatively narrow, it is not extended over C-F, from left to right in Text-fig. 1, but only covers over a small portion between D-E. As may be seen in Text-fig. 2, the mesentery D-E is shortened to a very great extent at its attachment on the rudiment D-E, and differential growth in width of this part of the mesentery results in the first half of the rudiment D-E being directly above the second half, *i. e.*, closer to the coelomic wall at which the mesentery originates. The attachment of the mesentery D-E rotates through an arc of 180° on the circumference of the rudiment, thus permitting the rudiment 1-3



TEXT-FIG. 2. Diagrammatic detail of portion of the regenerating tube at Stage 4, showing the manner in which it is suspended by the mesentery. The curved line marked 1-5 represents the course of attachment of the mesentery sheet to the dorsal surface of the coelom, and corresponds to the line D-E, Text-fig. 1. The regenerating tube B-F corresponds to the intestine path B-F of Text-fig. 1. The attachment of the sheet to the tube is indicated by the line along the upper surface of the tube. Numbers 1 to 5 along this line correspond to those on the body wall, and the lines of attachment are indicated, together with a phantom suggestion of the sheet of mesentery. The line of attachment of the sheet to the tube passes to the back of the tube in negotiating the loop so that point 4 is on the far wall of the tube. See text for full explanation.

to follow the free edge of the mesentery without passing through the sheet of mesentery supporting the rudiment 4-5 in Text-fig. 2.

As development proceeds, the mesentery B'-C', Text-fig. 1, widens to a great extent, allowing the rudiment which it supports to approach B-C, overlapping C-F. In Text-fig. 2, this development may be visualized if it be imagined that the rudiment B-C comes towards the viewer. The result at this angle of observation will be one of B-C in the foreground passing beneath E-F. Concomitant with this shift in spatial relationships, the mesentery 1-3 in Text-fig. 2 increases in width until it corresponds closely to the mesentery 4-5. This results in the attached rudiment 1-3 approaching the plane of the rudiment 4-5

so that these two parts now lie side by side instead of one on top of the other.

Another factor further complicates the shift of the rudiment from its path as shown in Text-fig. 2 to the normal course as represented in Text-fig. 1. The tubular rudiment shown in Text-fig. 2 does not extend along the mesentery as such beyond points B and F, but rather tapers abruptly between B-A and F-G, Text-fig. 1, to a fine solid rod, as described above in Stage 4. Concomitant with the development of the mesenteries allowing a shift in position of the rudiment, the development of the rudiment and its lumen progresses towards A from B and towards G from F. The effect is that of points B and F, Text-fig. 2, being pulled out along their axes,

shortening the rudiment C-E until rudiment D-E forms a sharp cusp where the rudiment changes direction, as described above in Stage 5. As both the rudiment and its supporting mesentery lengthen during further development, this cusp expands until it is a loop, as described in Stage 8. Not until regeneration is far advanced does the rudiment of this loop attain a length sufficient to follow the double spiral course of the normal intestine.

Histology

Although a rod-like intestine rudiment arises along the entire free edge of the mesentery, this rudiment develops a lumen between points B-F before there is any indication of a lumen at either extremity B-A or F-G. Histological cross-sections of this rudiment B-F support this observation and demonstrate the source of the lumen within the rod. At the extremities of this enlarged curved portion, the rudiment appears as a solid rod of connective tissue surrounded by a narrow band of epithelium only a few cells thick. However, in many places about the periphery of the rudiment in area B-F, a sequence of events resulting in the formation of a definitive lumen within the connective tissue of the rod may be followed in serial sections such as those shown in Plate I, Figs. 2, 3 and 4. A thickening of the epithelium results in an infolding of epithelial cells at the periphery of the rod. These folds deepen and are closed over at the periphery, leaving rings of epithelial cells surrounding a lumen within the rod itself, as seen in Pl. I, Fig. 4. A similar series of ridges and furrows runs lengthwise along the rod-like rudiment, the epithelium of the ridges thickening until two adjacent ridges close over a furrow, leaving a blind lumen lined with epithelial cells derived from the external epithelium of the rudiment. The change in size of this lumen through the rod of connective tissue may be traced in serial sections taken from area A-B of the regenerating intestine of an animal at Stage 5 (see Pl. II, Figs. 5, 6, 7 and 8). In Fig. 5 there are three individual lumina in the connective tissue core of the rudiment. It will be seen by following these lumina in the succeeding sections, Pl. II, Figs. 6, 7 and 8, that the right and left central ones are enlarging in a direction parallel to the long axis through the cord in one direction, while the lumen at the top of the section is enlarging in the opposite direction. The lumen at the top of the section in Pl. II, Fig. 5, may be seen to be reduced in size with thickened epithelial lining in Fig. 6, and in Fig. 7 there is no longer a lumen present, only a solid patch of epithelial cells. The opposite sequence may be seen in the left and right central lumen. In Fig. 6 there is a streak of epithelial

cells running from the lumen lining out into the surrounding connective tissue, and in Fig. 7 the lumen extends up into this streak. The sequence of events is clear. There are present in the rudiment a number of blind lumina running through the connective tissue of the rod formed as demonstrated in Pl. I, Figs. 2, 3 and 4. These extend in both directions along the axis of the rudiment, preceded by a solid core of actively multiplying epithelial cells. Thus numerous small lumina arise in this primary solid rod of tissue, destined to fuse into a continuous tube.

Sections of the rudiment A-B at Stage 6 (not illustrated) show it to be a simple tube with a single lumen running throughout. The walls of this tube are simply constructed, being of three layers: external, epithelium, generally one cell thick, surrounding the tube; connective tissue; and internal epithelium, one cell thick, lining the lumen. The internal epithelium closely resembles the external.

Sections made of the rudiment at G in a Stage 6 specimen show it to be largely a solid rod similar to that in the area B-F in Stage 4. There are some places at which there is a proliferation of epithelium at the periphery of the rod with subsequent folding and inclusion of a lumen but no well-defined, centrally located lumen is present.

To determine the extent of differentiation of the intestine wall, sections were made of the anterior dorsal intestine in area A-B from an animal which had regenerated for 25 days at the higher temperature range and whose gut was functional, at least mechanically, in ingesting and passing food matter. The histological picture of this animal was compared with sections made of the same area of the intestine of a normal animal. The normal picture corresponds closely to that described in detail by Jourdan (1883) Ludwig (1889-1892) and Bordas (1899). The lumen is lined by columnar epithelium capped by a hyaline cuticle. This epithelial layer projects into the lumen in numerous slender villi. Below the epithelial layer is a solid layer of connective tissue surrounded by a muscle band largely made up of circular fibers. On the outside of this muscular layer is another layer of connective tissue surrounded by a one-cell-thick sheath of cuboidal epithelium. The intestine wall of the 25-day-regeneration specimen appears strikingly similar to this. The columnar epithelium with its hyaline cuticle is raised in a few slender villi although these are not nearly as numerous as in the normal intestine. The greatest difference between the normal and regenerated intestine is the lack of a well-defined muscle layer. Only a few circular fibers can be discerned running through the connective tissue in the regenerated intestine.

DISCUSSION

It is apparent that, although the organ of Cuvier in *Actinopyga agassizi* contains the toxic factor holothurin, as reported by Nigrelli (1952), it plays no direct part in the secretion of this substance when the living animal is stimulated by rubbing. Further experimentation is necessary to determine whether or not there is some relationship between storage of the toxin by the organ of Cuvier and its secretion by the skin glands, although it has been demonstrated that there is no apparent effect on toxin secreted from the test of the animal over a period of five days following the removal of the Cuvierian organ. The single case of the survival of a pearl fish living inside a sea-cucumber after extensive poisoning indicates the possibility of a rather different role of the Cuvierian organ. It is conceivable that this organ, which contains the active toxic factor yet is not glandular in structure and apparently does not serve as a temporary storage reservoir in relation to the secretory cells of the test, acts in some way as a protection either on the respiratory system of the animal or on the coelomic fluid, possibly preventing poisoning of the sea-cucumber by its own toxin. If this were the case, a more direct relationship between the Cuvierian organ and evisceration might be hypothesized. The autotomy of the viscera would then be a reaction caused by the loss of the animal's protective mechanism, since the organ of Cuvier is eliminated prior to evisceration. Evisceration would be caused by exposure of the holothurian to its own toxin. On the basis of these preliminary studies, such a role of the Cuvierian organ can only be suggested as a possibility worthy of further investigation.

Although as described for *Stichopus regalis* (Bertolini, 1930), the regenerating intestine of *Actinopyga agassizi* arises along the entire free edge of the supporting mesentery, it has been found that in *A. agassizi* there is considerable variation in growth and development in different areas. The area B-F leads growth in widening of the mesentery, enlargement of the intestine rudiment and development of a lumen within the connective tissue core of the rudiment. This demonstrates initiation of regeneration and some control in differentiation derived locally from the mesentery rather than from the anterior broken tube of the alimentary canal. Not only is the early, solid, rod-like rudiment derived from the mesentery, but also the epithelial elements which invade the connective tissue core in the formation of a lumen originate locally from the simple external epithelium, rather than migrating into the formed rudiment from the lumen of the retained anterior fragment of the alimentary tract. This anterior fragment apparently has no

influence on the regeneration of the intestine. Its end opens into the coelom, closes in the early stages of regeneration, and opens again only after the rudiment has been developed into a tubular structure.

The factors which influence the higher speed of development in the area B-F cannot be clearly discerned at this time. Because of the important role of the mesentery, it is conceivable that in this area, in which a very large portion of the mesentery is gathered to support a relatively short portion of regenerating rudiment, one factor is simply that of quantitative influence of the large area of mesentery. In no other area throughout the track of the regenerating system is there even an approach to the ratio of mesentery to rudiment that exists in this area. It is in the initial stages of regeneration that the area B-F shows such marked advance over other areas. As the rudiment in this area lengthens, thereby reducing the mesentery-to-rudiment ratio, the areas A-B and F-H of the rudiment gain and eventually parallel the development of B-F.

Although the regeneration of the intestine has not been studied through to a condition closely resembling the normal one, regeneration appears to be sufficiently complete in the later specimens to permit ingestion and digestion of food matter. The histological picture of the regenerated intestine closely resembles that of the normal specimen in all but muscular development. It appears that further development consists chiefly of increasing length of the intestine. In addition, there must be further differentiation of musculature in the intestine walls and an increase in diameter before the regenerated organ closely resembles the normal.

SUMMARY

1. As has been found to be true of other aspidochirote holothurians, stimulation of evisceration can be easily effected in *Actinopyga agassizi* Selenka by a variety of conditions including high temperature and the presence of various metallic ions.
2. Preliminary experiments indicate no readily apparent relation between the organ of Cuvier and the ability of the living animal to release a potent quantity of the toxin, holothurin.
3. Histological sections of the organ of Cuvier show that it is not of tubular structure as described for other species, and bears no resemblance to a secretory organ. It gives the appearance rather of a storage tissue.
4. Subsequent to evisceration, the formation of an intestine rudiment takes place along the entire free edge of the remaining mesentery, but one area of growth proceeds at a faster rate than

all other areas. The greater length of the original mesentery and differential growth rates result in position shifts of the short, developing intestine rudiment.

5. A lumen is formed by proliferation of simple external epithelium at the periphery of the rudiment, followed by folding of the surface of the rudiment resulting in the inclusion of blind lumina within its connective tissue core. These lumina progress in both directions parallel to the axis of the rudiment, preceded by a mass of epithelial cells.

6. After 25 days of regeneration at 30° -32° C., the intestine is functional and histologically resembles closely the normal organ, although it is shorter and less convoluted and the walls lack normal musculature.

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EXPLANATION OF THE PLATES

PLATE I

- FIG. 1. Single strand ("tubule") of organ of Cuvier, in cross-section. 70X.
- FIG. 2. Cross-section of intestine rudiment showing proliferation of external epithelium at the top of the section, left of center. 70X.
- FIG. 3. The same area in the following section showing the proliferating epithelium thrown into deep folds. 70X.
- FIG. 4. A subsequent section showing the area of thickened epithelium cut off from the surface and surrounding a small lumen.

PLATE II

- FIG. 5. Transverse section of intestine rudiment A-B, showing three lumina. 16X.
- FIG. 6. A following section showing the same three lumina. The one at the top of the section narrows as it nears the blind end, the other two becoming larger. A cord of epithelial cells is seen proliferating from the left central lumen. 16X.
- FIG. 7. A following section showing closure of the lumen at the top of the section, only a core of epithelial cells remaining. The epithelial cord of the left central lumen now possesses a lumen of its own. 16X.
- FIG. 8. This section shows complete obliteration of the lumen at the top, while the two central lumina are larger still. This is interpreted as the two central lumina progressing lengthwise of the intestine in a direction toward the viewer, the top lumen progressing in the opposite direction. Top of Section: A few epithelial cells. Center: Right: Single-cell boundary of epithelial lining bulges into lumen.